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Are invertebrates relevant models in ageing research? Focus on the effects of rapamycin on TOR

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ABSTRACT

Ageing is the organisms increased susceptibility to death, which is linked to accumulated damage in the cells and tissues. Ageing is a complex process regulated by crosstalk of various pathways in the cells. Ageing is highly regulated by the Target of Rapamycin (TOR) pathway activity. TOR is an evolutionary conserved key protein kinase in the TOR pathway that regulates growth, proliferation and cell metabolism in response to nutrients, growth factors and stress. Comparing the ageing process in invertebrate model organisms with relatively short lifespan with mammals provides valuable information about the molecular mechanisms underlying the ageing process faster than mammal systems. Inhibition of the TOR pathway activity via either genetic manipulation or rapamycin increases lifespan profoundly in most invertebrate model organisms. This contribution will review the recent findings in invertebrates concerning the TOR pathway and effects of TOR inhibition by rapamycin on lifespan. Besides some contradictory results, the majority points out that rapamycin induces longevity. This suggests that administration of rapamycin in invertebrates is a promising tool for pursuing the scientific puzzle of lifespan prolongation.

Contents

1. Introduction

Ageing is represented by defective biological functions and increased susceptibility of the organism to death in response to gradual decline in physiological integrity (López-Otín et al., 2013), and the disease prevalence and ageing are highly correlated (Niccoli and Partridge, 2012; Townsend et al., 2012). In order to improve human health span and lifespan, it is a crucial step to understand the complex mechanisms underlying the ageing process. Why it is a fact that detailed understanding of molecular basis of ageing and ageing-related diseases is necessary. Within last decades, ageing research has gained momentum. López-Otin and colleagues reviewed in 2013 the molecular and cellular hallmarks of ageing formulated in nine categories including cellular senescence, mitochondrial dysfunction, deregulated nutrient sensing, loss of proteostasis, epigenetic alterations, telomere attrition, genomic instability, altered intercellular communication, and stem cell exhaustion.

Invertebrate model organisms are indeed important for ageing research. Already in late 1970s, studies in the filamentous ascomycete *Neurospora crassa* started to provide valuable information in ageing research. Series of studies analysed several age

Abbreviations: AMPK, AMP-activated protein kinase; IGF, insulin-like growth factor; PI3K, phosphoinositide 3-kinase; RHEB, RAS homolog enriched in brain; TOR, target of rapamycin; TORC, TOR complex; TSC1/2, tuberous sclerosis 1 and 2.

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modulating factors such as antioxidant enzymes (Munkres and Minssen, 1976) and dietary antioxidants (Munkres, 1976) in *N. crassa*. Additionally, an ageing-related increase in mitochondrial DNA reorganization was demonstrated in the filamentous fungi *Podospora anserina* (Belcour et al., 1981; Osíewacz and Esser, 1984). Later on, changes in mitochondrial DNA as crucial for the ageing process were confirmed in *Neurospora* species as well (Osiewacz, 1990; Griffiths, 1992). The first long-lived strains of the nematode *Caenorhabditis elegans* was isolated by Klass in 1983, and ageing research got a new invertebrate experimental target organism. Studies conducted with invertebrate models especially focus on two model organisms, *C. elegans* and the fruit fly *Drosophila melanogaster*. Besides those abovementioned models, yeast *Saccharomyces cerevisiae* has also been used in ageing research. The considerable evolutionarily conservation of the molecular pathways that play role in ageing and longevity provides relevant information in the process of uncovering the complexity of ageing even by using organisms positioned relatively low in the animal phylogenetic tree. This tool is deliberately selected for the ultimate benefit for revealing the phenomenon in humans.

This review aims to focus on Target of Rapamycin (TOR) signalling that is a key regulator of nutrient sensing in the cells, and the effect of its inhibition by rapamycin on ageing in invertebrate models. It illustrates the usefulness of invertebrate model organisms in ageing research and includes all data hitherto published with the effect of rapamycin on the ageing in invertebrates.

2. Rapamycin and target of rapamycin

Target of Rapamycin (TOR) is a serine/threonine kinase that belongs to the phosphatidylinositol kinase-related kinase family of proteins. TOR was initially discovered in the yeast *S. cerevisiae* (Heitman et al., 1991), in which it is encoded by two distinct genes, *TOR1* and *TOR2*. Role of the protein products of the genes are found to regulate various processes including cell cycle (Heitman et al., 1991), autophagy (Kamada et al., 2000), gene expression (Cardenas et al., 1999), and ribosomal biogenesis (Powers and Walter, 1999). In yeast, TOR exerts its activity in two distinct complexes, TOR complex 1 (TORC1) and complex 2 (TORC2) (Table 1). The elements of TORC1 are TOR1 or TOR2, KOG1, LST8 and Tco89p, whereas TORC2 contains TOR2, LST8, AVO1, AVO2, AVO3 and Bit61p (Inoki et al., 2005; Fig. 1a). In mammals, TOR is named as mammalian or mechanistic TOR (mTOR) (Table 1). It generates two functionally and structurally distinct complex, mTORC1 and mTORC2, while exerting its activity. The mTOR kinase is found in both mTOR complexes, as well as the complex-associated proteins Deptor, mLST8 (Widlund et al., 2013) and Tti1/Tel2 complex (Kaizuka et al., 2010). Additionally, mTORC1 contains PRAS40 and Raptor, and mTORC2 contains Rictor, mSIN1 and Protor-1 (Laplante and Sabatini, 2009; Fig. 1b). Besides mammals and yeast, TOR homologues have been identified in a variety of species including the filamentous fungi *P. anserina* (Pinan-Lucarré et al., 2006), the unicellular green algae *Chlamydomonas reinhardtii* (Crespo et al., 2005; Díaz-Troya et al., 2008), the flowering plant *Arabidopsis thaliana* (Menand et al., 2002), the protozoan *Trypanosoma brucei* (Barquilla *et al.,* 2008), the flatworm *Schmidtea mediterranea* (Peiris et al., 2012) the nematode *Caenorhabditis elegans* (Long et al., 2002), and the fruit fly *D. melanogaster* (Oldham et al., 2000) (Table 1). Several TOR complex associated proteins are conserved as well (Table 1).

Rapamycin, which is a macrolide, was first isolated from the soil bacterium *Streptomyces hygroscopicus* (Vézina et al., 1975). It has potent antifungal properties as well as immunosuppressive and anticancer properties (Vignot et al., 2005). Rapamycin exerts its activities by inhibiting mTOR cascade through binding of rapamycin to the FKBP-12 protein, and the rapamycin-FKBP12 complex binds to mTOR (Laplante and Sabatini, 2012). FKBP12-rapamycin complex only binds to Raptor not Rictor, therefore inhibits (m)TORC1 activity. Studies have shown that two TOR complexes differ depending on their sensitivity to rapamycin. Rapamycin inhibits TORC1 in *S. cerevisisae* potently, however it does not inhibit TORC2 (De Virgilio and Loewith, 2006). In mammals, mTORC1 is found to be sensitive to rapamycin treatment, but not mTORC2 (Widlund et al., 2013). However, one study has reported that prolonged rapamycin treatment inhibits mTORC2 activity (Sarbassov et al., 2006). In *T. brucei* TORC2 is sensitive to rapamycin whereas TORC1 is not (Barquilla et al., 2008). Previous studies showed that the presence of FKBP12 homologues does not always confer the inhibitory activity of rapamycin on TOR in plants. Imamura et al. (2013) identified several homologues of FKBP12 in unicellular red alga *Cyanidioschyzon merolae*, but presence of these homologues did not maintain the inhibitory activity of FKBP12-rapamycin complex on TOR. When *C. merolae* was treated with rapamycin, TOR was not inhibited, but when *S. cerevisiae* homologue of FKBP12 was expressed in *C. merolae*, TOR inhibition was observed. The FKBP12 homologues were also identified in *A. thaliana* that cannot interact with rapamycin and TOR. When *S. cerevisiae* homologue of FKBP12 was introduced to the *A. thaliana*, the interaction between TOR and ScFKBP12 was obtained in the presence of rapamycin (Sormani et al., 2007). Conversely, FKBP12 homologue of *C. reinhardtii* was shown to bind to TOR in the presence of rapamycin. Additionally, expression of rapamycin insensitive mutant of FKBP12 resulted in decrease in the inhibitory activity of rapamycin on cell growth compared to the wild-type and wild-type FKBP12 expressed in FKBP12 mutant strain (Crespo et al., 2005), suggesting that the activity and binding properties of rapamycin were species dependent in photosynthetic eukaryotes.

3. Upstream and downstream factors of TOR signalling

The TOR pathway activity in the cell is tightly regulated. The TORC1 activity is regulated by main factors as growth factors and hormones, nutrients, energy and stress such as genotoxic stress and hypoxia; yet, knowledge about the regulation of TORC2 is very limited (Wullschleger et al., 2006; Laplante and Sabatini, 2009; Zoncu et al., 2011). In this section, the TORC1 signalling pathway is the focus, because rapamycin is mainly regulating the TORC1 activity.

TORC1 acts as a wire and orchestrator between growth factor triggered signalling and processes such as autophagy and protein synthesis. In response to the binding of insulin or insulin-like growth factor (IGF) to its receptors, the insulin/IGF-PI3K-TSC axis regulates the TORC1 activity. The experiments conducted in *D. melanogaster* showed that binding of insulin or IGF to the receptors activates Phosphoinositide 3-kinase (PI3K) through phosphorylating it, which, in turn activates AKT. AKT inhibits the Tuberous sclerosis 1 and 2 (TSC1/2) complex by phosphorylation and inhibits the inhibitory activity TSC1/2 on RAS homolog enriched in brain (RHEB) GTPase. Active RHEB-GTP complex interacts with and activates TORC1 (Bier, 2005). Abovementioned pathway is conserved among many species including mammals, but TSC-Rheb-TORC1 axis of the pathway is neither present nor identified in several organisms including *A. thaliana, T. brucei* and *S. cerevisiae*, indicating alternative mode of actions.

Energetic status of the cell and nutrients also affect the TORC1 activity. AMP-activated protein kinase (AMPK) is one of the upstream regulators of TORC1 in response to the change in ADP/ATP and AMP/ATP ratios. Even a slight decrease in ATP level activates AMPK and leads to an increase in AMP and ADP production. By activation of AMPK it boosts the production of ATP by increasing the catabolic pathways while suppressing the anabolic pathways (Mihaylova and Shaw, 2011). Active AMPK either phosphorylates